Carnivory of *Byblis* revisited—A simple method for enzyme testing on carnivorous plants

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Introduction and theory

At the beginning of this year I had a conversation with Dr. Jan Schlauer from the University of Würzburg (Germany) concerning carnivorous plants (CPs). In the course of this he mentioned a report by Heslop-Harrison and Knox (1971) in which the authors described an easy method to detect enzyme activities in carnivorous plants using photographic film. This seemed so interesting to me that I decided immediately to try this out. The following day I spoke to an acquaintance in Switzerland (Luigi Francini), whose hobby is photography and who has a small photographic laboratory. I asked if he would be willing to develop some film for me. Luigi Francini (who also has some CP at home) was enthusiastic, and after a quick lesson about different materials used for photography the first tests started the following weekend.

The theory is quite simple. Digestive enzymes dissolve the gelatine layer of exposed film. The gel contains the finely distributed photosensitive chemicals such as halogenous silver crystals, pigments, etc., and is applied as an extremely thin suspension onto the plastic substrate which is mostly made from acetylcellulose or polycarbonate. Strips of processed but not fixed Kodalith ortho (ASA 12) black and white film are used for the test.

The enzyme production of the plants is triggered by a yeast solution. Twelve hours later some dexterity is required to fix the film strips onto the trapping leaves. After removal twenty-four hours later, the strips show a typical spotting if enzymes are present. Laboratory examination of enzymes (e.g. with protein gels) is very expensive whereas this procedure with customary films is a very simple alternative, is very low-priced (only 1 or 2 cents per test!), and is thus really interesting for use in private greenhouses and even for field tests if accordingly adapted.

Suitable photographic material and its application

As mentioned above, Kodalith ortho (black and white) film is used in the literature, mainly for lithography, microphotography, and other industrial purposes because it is very robust and provides a wide range of contrast. It is an industrial film which usually must be specially ordered by photo shops. Since it was not easily available, I decided to test some different types of customary material with varying gelatine layers.

Luigi Francini recommended three different types of ILFORD black and white films. The more photosensitive this film is, the thicker it is and the more coarse

grained its halogenous silver crystals are. A series of tests were used to show which material was most suitable. ILFORD FP4 (ASA 125), ILFORD HP5 (ASA 400) and ILFORD XP2 (ASA 400) were tested on *Drosera capensis*, *Drosera cuneifolia* and *Byblis liniflora*. Instead of the method described in literature, I used only exposed (and not additionally processed and unfixed) material because it was my idea to find a test which was practicable and easy as possible. To stabilise the results on the strips after the exposure to the plants for further photographs, they were processed but not fixed by Luigi Francini and afterwards showed finer and durable details.

Significantly clearer results could be seen on the two coarse grained ASA 400 negatives (which had the thicker gelatine layer). Some looked like 'X-ray' images so the test plant could often be identified, whereas the FP4 ASA 125 was much lower in quality (where in one case a *Drosera cuneifolia* fed with a mosquito larva digested a 2 mm hole into the gel). Even after processing, the negatives of the sundews showed only a few, poor spottings and *Byblis liniflora* showed nothing. This result was surprising to me, because the Kodalith ortho film used in literature has only a sensitivity of ASA 12.

The negatives of the HP5 and XP2 showed the enzyme activities exactly the other way around, meaning black and white in reverse. Held against the light, the XP2 showed the clearest outlines of glue drops and (in excellent cases) even of the tentacles, so that it displayed nearly a complete image of the trapping leaf. The colour of the whole leaf area is light to pale grey, perhaps caused both by soaking the gelatine or by leaching soluble parts of the layer by humidity during the exposure to the plant. It was not a result of enzyme activity because the same effect also took place with pure water. More interesting were the black centres with clear perforations. Here the gel was dissolved by enzyme. The light shining through those holes in the layer and the black zones around provide a good contrast. For this reason the ILFORD XP2 was chosen as the most suitable film.

To expose the film I pulled it out of the roll and cut it into 1.0 to 2.5 cm wide strips, according to the size of the plant to be examined. To mark the film type and assigned plant durably (and in a way that would not be obliterated by the processing-bath) I cut different notches into the strips. Dates were conscientiously recorded. As mentioned above, correct fastening of the film strips on the trapping leaves of CPs needs a little imagination to achieve good "illustrations!"

Choice of suitable CPs

To check the methods, I chose adult plants of *Drosera capensis* and *Drosera cuneifolia* as references, because the enzyme production of sundews (*Drosera*) has been thoroughly examined and proven. In addition I used a one-year old rainbow plant (*Byblis liniflora*) of which the production of enzymes was still unclear. Bruce (1905) did some investigations on *Byblis gigantea* growing near Perth in Western Australia that showed positive results, but did not examine *Byblis liniflora*.

This question of carnivory for *Byblis liniflora* was of special interest to me since I had visited some north Australian habitats with my wife Irmgard, and we demonstrated a mutualism involving Capsid bug species (Miridae) and *Byblis liniflora* on our video (Hartmeyer & Hartmeyer 1995). That symbiosis showed much similarity with the two species of the South African *Roridula* and their *Pameridea*-bugs, which are also true bugs belonging to the Miridae. We observed this symbios for six years in our living-room in Weil am Rhein, and got some nice video shots which can be seen on our video (Hartmeyer & Hartmeyer 1994). However, a number of experts do not include *Roridula* with CPs because it shows no enzyme production. If the tests proved that *Byblis* does not produce enzymes and also shows the same sym-

biosis with closely related Capsid bugs, a new discussion by the experts about the assignment of the Rainbow Plant as a CP would be necessary.

This line of thought is particularly interesting because of the current discussion about the plumbagin branch of CPs. Plumbagin is a compound present in the "old" CP families Droseraceae, Drosophyllaceae, Nepenthaceae and Dioncophyllaceae, in which all species are able to produce proteolytic enzymes (Schlauer 1997). Observing the 'plumbagin-free' branches, only the two "modern" CP families Cephalotaceae and Lentibulariaceae show this uniform behaviour. All other families have at least members which do not produce enzymes. Even the Sarraceniaceae, which are the stars of nearly every CP book, contains such plants, e.g. *Darlingtonia* and several species of *Heliamphora*. Interestingly, without any own enzymes those plants make numerous bacteria and arthropods do the digesting. This was confirmed recently by Ellis & Midgley (1996), who did large-scale tests with isotope-labelled (^{15}N) flies on *Roridula* associated with *Pameridea* bug populations. They observed that the isotopes sucked out of the prepared insects by the resident Capsid bugs reached the plant leaves via the feces dropped on the leaves, and were being absorbed probably through stomata. So it has been proven that *Roridula* is able to benefit directly from the wide selection of prey sticking to its leaves and not only via soil fertilisation by the natural decay of its prey.

The results of further enzyme tests can contribute to the clarification of the questions of carnivory—whether in the future some of those plants assigned today as true CPs (e.g. *Byblis*, *Darlingtonia*, *Heliamphora*) may not be classified carnivorous anymore, or if the definition for a true CP must be reformed to allow for enzyme-free, and passive predigestion by symbiotes.

Enzyme production

In the literature, a yeast solution is used to trigger the enzyme production by the plants. Some tests with pure yeast, as well as solutions diluted by water, all showed a clear reaction from *Drosera*, which meant the tentacles and sometimes the whole leaf started to surround the treated spot.

If the solution was too concentrated coarse lumps appeared on the traps. This resulted in low quality illustrations of the tentacles and glue drop contours on the film. Pure yeast and dried mosquito larvae (which showed the strongest reaction!) yielded similar results.

The best results were achieved by a 10% solution of yeast in water. Depending on the trap size, one to three drops were applied using a small pipette. This procedure resulted in the most detailed outlines and was digested by all the plants without problems.

Preparing the series of tests

The tests described above yielded a second result concerning *Byblis* that I did not expect to be so unequivocal. On ASA 400 material all *Drosera* appeared as clear positive, but no *Byblis* showed enzyme production. To explore this result (which is surprising for what is usually considered a true CP), and to further test the whole laboratory procedure, I decided to follow the first tests with the following experiments:

a) Nine different *Drosera* species as additional positive references. See Table 1
b) Two specimens of *Roridula dentata* were tested three times, as negative references.
c) Two specimens of *Byblis liniflora* were tested four times to confirm the results.

d) *Drosera capensis aliciae* was tested twice to get a double-determination for this hybrid.
e) *Drosera macrophylla* was fed with 1.5 mosquito larvae to check the strong enzyme reaction.
f) The exposure time on the plants were increased from 24 to 32 hours to improve the quality of the images.

Results

The results of the new battery of experiments are as follows.
a) All nine *Drosera* species showed unequivocal positive results.
b) Ellis and Midgley (1996) used the Kodalith ortho film method by Heslop-Harrison and Knox (1971) for their investigations at the University of Cape Town. The negative results on *Roridula* were confirmed. More surprising were the holes in the gelatine layer caused by the *Roridula* leaf glands. On closer examination it became clear that the highly adhesive caoutchouc in the glue was damaging the gelatine layer. Every drop tore off parts of the gelatine when the strip was removed. So no enzyme activity was detected on *Roridula*. Under high magnification, the margins of the holes were clearly different from holes caused by proteolytic reaction.
c) All tests on *Byblis liniflora* were negative.
d) The two tests on *Drosera capensis aliciae* proved the reproducibility of the method.
e) Again the dried mosquito larvae triggered the strongest enzyme production.

The chosen ILFORD XP2 material worked as expected and the increased exposure time also increased the illustration quality notably. The results were detailed images with a charming X-ray appeal even *without using processed and unfixed film*. But if processed, finer details of the images appeared, the durability of the negatives improved, and magnifications could be made. For documentation the strips were photographed on colour slide film, because the different grey-shades are reproduced much better than on black and white film, as Luigi Francini found out.

Conclusions

Using species of *Drosera* as a reference is recommended. The results of the tests on *Byblis liniflora* are a real surprise. Further experiments on other CP species are in preparation, but to describe them would go beyond the scope of this article. The described tests are not only very inexpensive (one film roll allows approximately fifty tests!), they are very easy to perform, show good reproducibility and are absolutely harmless for the examined plants. In fact, the more yeast or mosquito larvae are given to the plants, the more they benefit. These attributes make the test highly interesting for private CP collectors, and so (I hope) promise a flood of new test results if published for a wider, CP-interested, public. Easy field experiments are possible if the tested plants are sheltered against the weather during the exposition time.

A highlight of this study is the following: If a sensitive (e.g. ASA 400) conventional black and white film is used in the above described way, a reproducible and detailed low cost test without any chemical bath is possible!

Last but not least I would like to express my gratitude to Luigi Francini for his friendly support and professional treatment of all photographic works. And I wish success for everybody who tries the test, because I'm sure we can await further surprises on this subject.

Table 1: Test Results

Plant[a]	Appearance of the processed negatives	Enzyme activity[b]
B. liniflora[c] (15-19 cm tall)	Faint light outlines of the glue-drops were visible. No dark centres or perforation appeared.	—
D. adelae (15 cm)	Clear light outlines contrasted with dark centres. The gelatine layer was partially perforated.	++
D. aliciae (4.5 cm)	Clear light outlines contrasted with dark centres. The gelatine layer had no perforations.	+
D. browniana (6 cm)	Clear light outlines contrasted with dark centres. Big (1-2 mm) holes occurred in the gelatine layer.	+++
D. capensis (14 cm)	Clear outlines contrasted with dark centres. The gelatine layer was partially perforated.	++
D. capensis × *aliciae*[d] (10 cm)	Clear light outlines contrasted with dark centres. The gelatine layer was strongly perforated.	+++
D. capensis × *aliciae*[e] (10 cm)	Clear light outlines contrasted with dark centres. The gelatine layer was strongly perforated.	+++
D. cuneifolia (6 cm)	Clear light outlines contrasted with dark centres. The gelatine layer was partially perforated.	++
D. macrophylla[f] (9 cm)	Clear light outlines contrasted with dark centres. Big (up to 10 mm!) holes inside the gelatine layer.	+++
D. prolifera (9 cm)	Clear outlines contrasted with dark centres. Gelatine layer was strongly perforated.	+++
D. stolonifera (15 cm, 11 cm tall)	Clear outlines contrasted with dark centres. The gelatine layer was partially perforated.	++
R. dentata[g] (18 cm, 28 cm tall)	No light contours or dark centres. Many small dot-like holes were visible inside the gelatine layer[h].	—

Table 1: Results of the enzyme experiments performed 17 January 1997—26 January 1997.
(a) Measurements are for the plant's diameter, unless otherwise noted.
(b) Symbols used:
- — Enzyme test negative
- + Enzyme test positive (Gelatine layer shows dark centres without perforation).
- ++ Enzyme test positive (Gelatine partially perforated).
- +++ Enzyme test positive (Gelatine layer strongly perforated, possibly big holes).

(c) Four separate tests were made.
(d) Test 1.
(e) Test 2.
(f) Also fed 1.5 mosquito larvae.
(g) Three separate tests were made.
(h) Caused by tearing of the emulsion and not by enzymes (see the Results section)

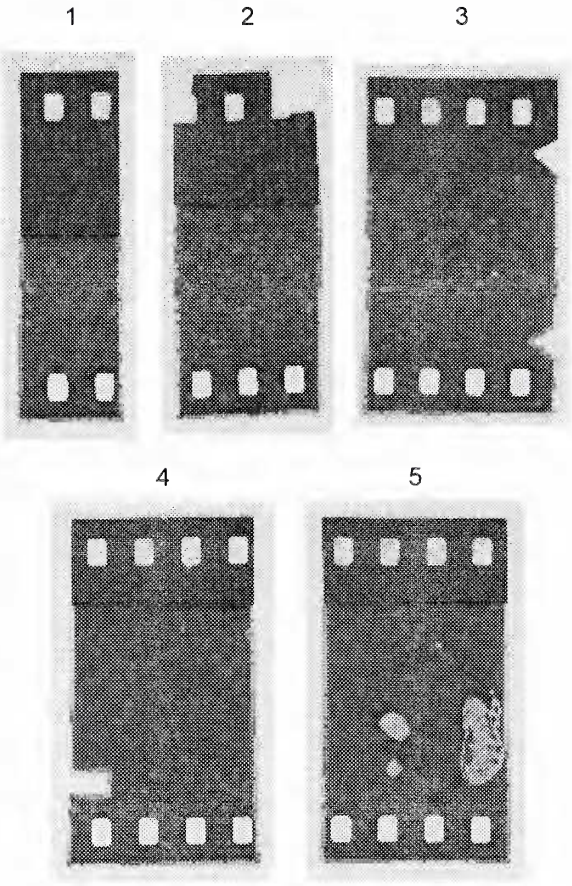

Figure 1: The film strips used to detect enzyme activity in various CP species.

Strip #	notches on the strips	species tested
1	none, narrow strip	*Byblis liniflora*
2	notch on perforation-side	*Roridula dentata*
3	two triangular notches	*Drosera capensis* × *aliciae* (Test 1)
4	one narrow notch	*Drosera adelae*
5	none, broad strip	*Drosera macrophylla*

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